

# Copepod grazing impact on the trophic structure of the microbial assemblage of the San Pedro Channel, California

ASTRID SCHNETZER\* AND DAVID A. CARON

DEPARTMENT OF BIOLOGICAL SCIENCES, UNIVERSITY OF SOUTHERN CALIFORNIA, 3616 TROUSDALE PARKWAY, LOS ANGELES, CA 90089-0371, USA

\*CORRESPONDING AUTHOR: astrids@usc.edu

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*In August 2002 and March 2003 the trophic structure of the microbial assemblage from the San Pedro Channel, California was studied following the experimental alteration of the number of copepods. Changes in the abundance/biomass of microorganisms <80 µm during 3-day incubations were monitored in (i) the absence of metazoa >80 µm, (ii) the presence of natural abundances of metazoa and (iii) the presence of an elevated number of copepods. Prokaryotes and small-sized eukaryotes (<4 µm) dominated plankton biomass during both experimental months. Diatoms numerically dominated the 10–80 µm plankton in August 2002, but ciliate and heterotrophic dinoflagellate biomass generally exceeded diatom biomass on both dates. Ingestion of protozooplankton (predominantly ciliates) contributed substantially to copepod daily carbon rations. The adult copepod assemblage removed 4.6 and 36% per day of the microzooplankton standing stocks (10–80 µm size fraction) in August and March, respectively. Elevated copepod grazing pressure on protozooplankton resulted in increased biomass of nanoplankton (<5 µm) presumably via a trophic cascade. Accordingly, the copepod–protozoan trophic link appears to be a key factor structuring the planktonic microbial assemblage in the San Pedro Channel.*

## INTRODUCTION

Copepods directly affect the standing stocks and community composition of prey assemblages through their grazing activities and may also impact the biomass and diversity of populations at lower trophic levels via trophic cascades (Fessenden and Cowles, 1994; Pace *et al.*, 1998; Katechakis *et al.*, 2002; Sipura *et al.*, 2003; Broglio *et al.*, 2004). The latter effects, in part, are mediated by the removal of intermediate consumers such as protozooplankton, which can decrease grazing pressure on prokaryotes and/or small-sized protists thereby allowing proliferation of these minute plankton (Jürgens and Güde, 1994; Lonsdale *et al.*, 1996; Calbet and Landry, 1999; Katechakis *et al.*, 2002).

It is assumed that trophic cascades may be particularly strong in oligotrophic waters where the dominant phytoplankton are too small (<5 µm) to be directly consumed by most mesozooplankton. In these situations, copepods could be expected to rely heavily on hetero-

trophic protists (protozoa) as their primary prey (Hansen *et al.*, 1994; Atkinson, 1996; Ptacnik *et al.*, 2004). The copepod–protozoan trophic link can also play a fundamental role in structuring planktonic microbial assemblages in general because copepods often show a preference for heterotrophic prey even when suitably-sized phytoplankton are available (Dam *et al.*, 1993, 1995; Turner *et al.*, 2001; Broglio *et al.*, 2004; Calbet and Saiz, 2005), and the nutritional value of protozoa appears to be high (Stoecker and Capuzzo, 1990; Klein Breteler *et al.*, 1999). Nevertheless, many studies have noted that trophic cascades are often undetectable beyond one trophic level (Nielsen and Kiorboe, 1994; Lonsdale *et al.*, 1996; Levinsen *et al.*, 2000; Katechakis *et al.*, 2002; Sipura *et al.*, 2003). One factor that may weaken the effects of trophic cascades as they propagate through the food web is the complexity of trophic relationships within natural assemblages (Hairton and Hairton, 1997; Polis *et al.*, 2000).

*This paper is one of six on the subject of the role of zooplankton predator–prey interactions in structuring plankton communities.*

The goal of this study was to determine the grazing impact by the copepod community on the trophic structure of the microbial assemblage in the San Pedro Channel off the southern Californian coast. This environment represents a temporal (seasonal) and spatial mosaic of moderately productive to oligotrophic coastal ecosystems (Hickey, 1992). Experiments were carried out to examine the role that protozoa play in copepod diets and the effect that the consumption of protozoa by copepods had on the planktonic community during August 2002 and March 2003. The number of copepods was experimentally manipulated and the impact of this manipulation on natural prey populations was examined by following changes in abundance and biomass of phototrophic and heterotrophic microorganisms <80  $\mu\text{m}$  in size during 3-day incubations. The copepod assemblage significantly impacted biomass of microzooplankton (10–80  $\mu\text{m}$  size range) on one of the sampling dates (36% of standing stock removed per day) and demonstrated a strong feeding preference for ciliates over heterotrophic dinoflagellates.

## METHOD

### Study site and experimental approach

Seawater for predation addition/exclusion experiments in summer 2002 (August) and spring 2003 (March) was obtained from the central San Pedro Channel approximately 20 km from Long Beach/San Pedro, California, USA (33°33' N and 118°24' W). This location is the study site of an ongoing 'Microbial Observatory', which focuses on the microbial diversity (bacteria, archaea and protists) in the coastal waters of the eastern North Pacific. Complementary data on water column properties are collected monthly as a part of the San Pedro Oceanographic Time-series Program (SPOT; <http://wrigley.usc.edu/research/spot.html>) and provide the oceanographic context at this site. Seawater for the experiments was obtained by inverting 50 L carboys with their covers off under the surface of the water and opening the spigot to allow the carboys to fill with seawater. Copepods were collected gently by conducting net tows (200- $\mu\text{m}$  mesh) in the upper water column (5–10 m). Seawater samples and plankton tows were brought within 1 h to the Wrigley Marine Science Center (WMSC) on Catalina Island where the treatments were prepared and the experiments were carried out.

Whole seawater (WSW) from the 50 L carboys was prefiltered through an 80- $\mu\text{m}$  Nitex mesh to fill six 4-L incubation bottles (exclusion treatment). The 80- $\mu\text{m}$  mesh retained copepods, most copepodites and nauplii, while only negligible numbers of large protists were

caught on the mesh (a few larger foraminifera and acantharea, the largest tintinnids, dinoflagellates or occasionally a diatom chain). The exclusion treatment provided a measure of changes in the planktonic microbial assemblage in the absence of metazoan grazers. Fifteen 4-L bottles were also filled with WSW treatment (unfiltered) to examine changes within the microbial assemblage in the presence of metazoa >80  $\mu\text{m}$ . Three WSW bottles were processed immediately as described below for measurements representing starting conditions of the experiments ( $T_0$ ). Six WSW bottles remained unamended, while copepod numbers in the remaining six bottles were increased in order to examine enhanced copepod grazing pressure on the microbial assemblage (addition treatment). To accomplish this, adult copepods were picked from the plankton tows, rinsed in 0.2  $\mu\text{m}$  filtered seawater and 40 (August 2002) or 45 (March 2003) healthy specimens were added to each bottle.

Immediately after preparing all treatments the contents of three bottles that contained WSW were pre-screened using an 80- $\mu\text{m}$  Nitex mesh (to remove metazoa). Subsamples of the filtrate were collected to determine abundances of micro-, nano- and picoplankton and chlorophyll *a* (Chl *a*) concentrations at the onset of the experiments ( $T_0$ ). The metazoa were rinsed from the 80- $\mu\text{m}$  mesh using filtered seawater and preserved in Formalin (5% final concentration) for counting, sizing and identification. Adult copepods were identified according to Dawson and Knatz (Dawson and Knatz, 1980). The remaining 18 bottles were incubated in an outdoor seawater tank at WMSC with ambient subdued light (~36% of incident light). Incubation temperatures varied only slightly over the duration of each experiment and averaged  $20.5 \pm 0.5^\circ\text{C}$  in August and  $17 \pm 0.9^\circ\text{C}$  in March. Upper water column temperatures (integrated over 0–10 m) at the collection site in the San Pedro Channel were slightly lower for the same months at 19.7 and 15.0°C, respectively. Three bottles from each treatment (exclusion, WSW, addition) were sampled as described above after 24 h of incubation and again after 72 h.

### Plankton enumeration and biomass calculations

Changes in plankton community structure due to copepod predation were examined by comparing the abundance/biomass of phototrophic and heterotrophic plankton <80  $\mu\text{m}$  in size among the different treatments over the time course of the experiments. Protists in the 10–80  $\mu\text{m}$  size fraction were enumerated by inverted light microscopy using standard settling techniques for duplicate samples (100 mL) preserved with either Lugol's solution (10% final concentration) or formalin (1% final

concentration). The use of formalin allowed heterotrophic and phototrophic cells to be distinguished based on the autofluorescence of photosynthetic pigments while samples preserved with Lugol's solution allowed better visualization of some taxa by inverted light microscopy (Utermöhl, 1958; Sherr *et al.*, 1993). Protists were assigned to broad taxonomic groups based on morphological examination (diatoms, ciliates, dinoflagellates and other flagellates). Biovolume (BV) was calculated from geometric shapes that best approximated cell shape for 40–80 individual organisms for each protistan group in each of the two experiments (sizes were averaged across all time points and treatments). Average BVs were then converted into carbon biomass by applying published conversion factors for ciliates ( $0.19 \text{ pgC } \mu\text{m}^{-3}$ , Putt and Stoecker, 1989), diatoms and dinoflagellates ( $\text{pgC } \mu\text{m}^{-3} = 0.288\text{BV}^{0.811}$  and  $\text{pgC } \mu\text{m}^{-3} = 0.76\text{BV}^{0.819}$ , respectively, Menden-Deuer and Lessard, 2000) and other flagellates ( $\text{pgC } \mu\text{m}^{-3} = 0.433\text{BV}^{0.863}$ , Verity *et al.*, 1992).

Heterotrophic and phototrophic nanoflagellates  $<10 \mu\text{m}$  in size were enumerated using epifluorescence microscopy on samples (50 mL) preserved with formalin (1% final concentration), stained with 4',6-diamidino-2-phenylindole ( $25 \mu\text{g mL}^{-1}$  final concentration) and filtered onto  $0.8\text{-}\mu\text{m}$  black polycarbonate filters (Sherr *et al.*, 1993). As described for microplankton, heterotrophs and phototrophs were distinguished based on the autofluorescence of photosynthetic pigments. Biomasses for these assemblages were calculated using published BV-carbon conversion factors (Verity *et al.*, 1992).

Phototrophic protists  $<2.5 \mu\text{m}$  in size, heterotrophic picoplankton (bacteria + archaea) and phototrophic prokaryotes (*Synechococcus* spp. + *Prochlorococcus* spp.) were enumerated by flow cytometry (FACSCalibur, Becton Dickinson) from samples preserved with formalin (1% final concentration) and frozen in liquid nitrogen until analyzed (Olson *et al.*, 1990a,b; Rose *et al.*, 2004). An average cell size for the phototrophic protists ( $<2.5 \mu\text{m}$ ) was determined based on forward scatter measurements in relation to forward scatter regression curves derived from fluorescent standard-sized beads. A spherical cell shape was assumed for calculating carbon biomass using published BV-carbon conversion factors (Verity *et al.*, 1992). A cell carbon value of  $0.015 \text{ pg C cell}^{-1}$  was applied for heterotrophic picoplankton (bacteria + archaea) (Caron *et al.*, 1995). Carbon biomass for *Synechococcus* spp. and *Prochlorococcus* spp. was directly calculated using cell carbon values of  $0.203$  and  $0.031 \text{ pg C cell}^{-1}$ , respectively (Heldal *et al.*, 2003).

Samples for pigment analyses were collected on GF/F Whatman filters and stored at  $-20^\circ\text{C}$  until analyzed. Chl *a* concentrations were measured using a Turner TD 700

fluorometer according to procedures in Parsons *et al.* (Parsons *et al.*, 1984a).

### Copepod ingestion rates

Copepod ingestion rates for ciliates and heterotrophic dinoflagellates ( $\mu\text{g C copepod}^{-1} \text{ day}^{-1}$ ) were calculated from differences in the rates of change of prey biomass among the treatments without metazoa (exclusion treatment), with natural (WSW), and elevated (addition) abundances of copepods (Frost, 1972). Decreases in biomass during the 3-day incubations were assumed to be the result of copepod predation (i.e. losses due to ciliates and heterotrophic dinoflagellates preying on each other were considered negligible). Copepod ingestion rates for diatoms, phototrophic dinoflagellates and 'other flagellates' ( $10\text{--}80 \mu\text{m}$ ) were not estimated because these microplankton assemblages may have been heavily preyed upon by the entire heterotrophic community (protozooplankton and copepods) and thus the impact of copepods alone could not be distinguished.

Copepod dry weights (DWs) for both experiments were measured for groups ( $n = 4$ ) of 80 copepods, which were picked from the tows and stored frozen. The samples were thawed, dried at  $60^\circ\text{C}$  for 24 h and weighed. DWs were converted into carbon weight by assuming carbon weight =  $0.4 \times \text{DW}$  (Parsons *et al.*, 1984b).

Copepod ingestion rates for ciliates and heterotrophic dinoflagellates for the 72-h incubations were compared to estimates of basic respiratory requirements for copepods based on empirical relationships published in the literature (Ikeda and Motoda, 1978; Hirst and Shearer, 1997). These relationships describe the correlation between copepod respiration and average copepod body weight and habitat temperature as

$$R = aW^b \quad (1)$$

where  $R$ , respiration rate ( $\mu\text{L O}_2 \text{ day}^{-1}$ );  $W$ , average copepod DW ( $\mu\text{g}$ );  $a$ ,  $10^{(0.02538T - 0.1259)}$ ;  $b$ ,  $-0.01089T + 0.8918$  and  $T$ , temperature (C). Oxygen values were converted into respiratory carbon ( $\mu\text{g C copepod}^{-1} \text{ day}^{-1}$ ) after calculating respiration rates ( $\mu\text{L O}_2 \text{ day}^{-1}$ ) and assuming a respiratory quotient of 0.8. These values were compared to carbon ingestion rates derived from each of the incubation experiments.

## RESULTS

### Initial microbial abundances and biomass

Microbial assemblages at the onset of the two experiments in surface waters of the San Pedro Channel showed a typical pattern of increasing abundances with

Table I: Cell abundances (cells mL<sup>-1</sup>) and carbon biomass (µg C L<sup>-1</sup>) in initial seawater samples (T<sub>0</sub>) in August 2002 and March 2003

	August 2002		March 2003	
	Cells mL <sup>-1</sup>	µg C L <sup>-1</sup>	Cells mL <sup>-1</sup>	µg C L <sup>-1</sup>
Ciliates	5.2 (±3.5)	6.50 (±3.2)	6.9 (±4.1)	17.80 (±12.6)
Heterotrophic dinoflagellates	6.0 (±3)	3.80 (±2)	1.9 (±0.9)	4.80 (±0.3)
Phototrophic dinoflagellates	8.4 (±4.5)	4.10 (±0.4)	1.7 (±0.8)	2.50 (±2.6)
Diatoms	68.5 (±11.8)	5.60 (±0.9)	10 (±1.8)	0.14 (±0.05)
Other flagellates (10–80 µm)	26.9 (±2.9)	1.70 (±1.7)	5.4 (±3.6)	0.22 (±0.2)
Nanophototrophs (2.5–10 µm)	2056 (±523)	10.00 (±3)	4461 (±1194)	36.00 (±10)
Nanoheterotrophs (<10 µm)	1592 (±941)	11.00 (±7)	4811 (±1059)	63.00 (±14)
Phototrophic eukaryotes (<2.5 µm)	6767 (±1678)	4.50 (±1.1)	31463 (±3206)	19.50 (±2)
Heterotrophic picoplankton	5,327,534 (±1,135,313)	79.91 (±17.03)	1,898,178 (±234916)	28.47 (±3.52)
Phototrophic prokaryotes	72,126 (±6452)	14.50 (±1.27)	25,167 (±2628)	4.93 (±0.47)

Seawater was prefiltered through an 80-µm Nitex mesh to exclude metazoan grazers before cell abundances and carbon biomass were estimated (see Method section for details). Means derived from three samples are listed (±SD).

decreasing cell size (Table I). Overall, cell abundances ranged from <10 mL<sup>-1</sup> for some microplanktonic assemblages to >10<sup>6</sup> mL<sup>-1</sup> for heterotrophic picoplankton (heterotrophic bacteria/archaea). In general, biomass values among the plankton categories were more equitable. Total microbial biomass (<80 µm) was 142 µg C L<sup>-1</sup> in August 2002 and 177 µg C L<sup>-1</sup> in March 2003. Prokaryotes (cyanobacteria, bacteria + archaea) constituted most of the biomass in August (67% of total carbon) whereas eukaryotes <10 µm in size (mainly phototrophic and heterotrophic flagellates) were major contributors in March (67% of total carbon; Table I). Eukaryotes <10 µm cell size (phototrophs + heterotrophs) averaged 3.9 µm (equivalent spherical diameter) in August and 3.4 µm in March. Thus, microorganisms <5 µm constituted approximately 85% of the total microbial biomass at the time of both experiments.

The summed biomass of microorganisms 10–80 µm in size was roughly equivalent in August 2002 and March 2003 (22 vs. 25 µg C L<sup>-1</sup>) but composition within this size fraction differed on the two sampling dates (Table I). Phototrophs contributed 49% of the biomass in this size fraction during August compared to 11% in March. Diatoms were the most abundant microplankton in August 2002 with an average of 69 cells mL<sup>-1</sup>, with *Pseudo-nitzschia* spp. constituting ~70% of all diatoms. Diatom abundances in March 2003 were only 8 cells mL<sup>-1</sup> and *Pseudo-nitzschia* spp. were rare. Dinoflagellates constituted 36% of the total biomass of the 10–80 µm size fraction in August and 29% in March, with roughly equal proportions of phototrophs and heterotrophs (Table I). Ciliate abundances on both sampling dates

were similar (5.2 and 6.9 cells mL<sup>-1</sup> in August and March, respectively) but average ciliate cell volume during March was 2.5 times the average ciliate cell volume during August. Thus, ciliate carbon contributed 30% of the total biomass in the 10–80 µm fraction during August but 70% of the biomass in this size fraction during March. Aloricate oligotrichs dominated the ciliate assemblage on both dates.

### Natural copepod abundance and taxonomic composition

Abundances of adult copepods in the San Pedro Channel differed substantially between the two sampling dates. Abundances of <1 copepod L<sup>-1</sup> were observed in August 2002 and 8 copepods L<sup>-1</sup> in March 2003 (Fig. 1, Time = 0 h for WSW). *Clausocalanus* spp. and *Oithona* spp. (mainly *Oithona similis*) were the most common copepods in August, while *Acartia* spp. (*Acartia bifilosa*, *Acartia tonsa* and *Acartia* spp.) also contributed significantly to the copepod assemblage in March. Common but subdominant members of the copepod community in both months were *Paracalanus* spp., *Calocalanus* spp. and *Oncaea* spp. Large specimens of *Calanus* spp. were occasionally encountered on both dates. Total copepod length of the adults averaged 827 ± 168 µm and DW was 7.3 ± 3.6 µg individual<sup>-1</sup> in August 2002. The corresponding values in March 2003 were 1190 ± 234 µm and 17.7 ± 12.5 µg DW individual<sup>-1</sup>.

Initial abundances of copepod nauplii and copepodites in August were both <1 individual L<sup>-1</sup>. Respective values for March were <1 and ~3 individuals L<sup>-1</sup> (Fig. 1). Metazoan zooplankton other than copepods (the larvacean



**Fig. 1.** Number of copepods, copepodites and nauplii (individuals  $L^{-1}$ ) in three different experimental treatments during August 2002 and March 2003. The treatments were (i) exclusion treatment: seawater was prefiltered through an 80- $\mu m$  mesh to exclude metazoan predators; (ii) whole seawater (WSW) treatment, in which unfiltered, unamended seawater with natural abundances of metazoan predators was examined and (iii) addition treatment; seawater in which the number of copepods was increased above ambient abundances. Means derived from three bottles are shown with their standard deviation. # indicates that the values are significantly different from the values at a previous sampling point for the same treatment ( $P < 0.05$ ,  $t$ -test). Arrows mark the efficient removal of adult copepods due to prefiltration with an 80- $\mu m$  mesh compared to the WSW incubation bottles. Note differences in the scale for y-axis.

*Oikopleura* sp., small medusae and chaetognaths) occurred occasionally in some of the bottles at much lower abundances than copepods. They were effectively removed by prefiltration through the 80- $\mu m$  mesh in the exclusion treatment.

### Copepod addition/exclusion treatments

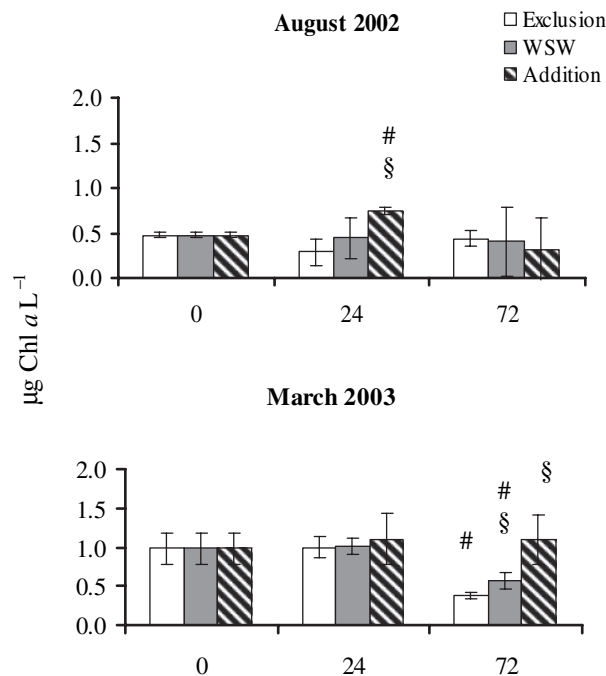
Adult copepods were effectively removed by prefiltration through an 80- $\mu m$  mesh from the exclusion treatment bottles (arrows in Fig. 1,  $T_0$ ). In August 2002, the number of copepods in the addition treatment was increased by 14-fold compared to natural abundances, while copepod additions in March 2003 resulted in an increase of copepods by a factor of 2.4 (Fig. 1,  $T_0$ , addition treatment vs. WSW).

In August, the number of copepods in the addition treatment was significantly higher with 11 individuals  $L^{-1}$  at the beginning of the experiment compared to  $\sim 8$  individuals  $L^{-1}$  after 24 and 72 h of incubation ( $P < 0.05$ ,  $t$ -test; Fig. 1). In the WSW treatment the number of adult copepods at 24 h was significantly lower than at 72 h ( $P < 0.05$ ,  $t$ -test; Fig. 1). No significant differences in abundances of adult copepods were observed over

the duration of the March experiment in any of the treatments (Fig. 1). The number of nauplii and copepodites generally varied over the course of both experiments (Fig. 1). These changes presumably reflected the hatching of eggs and development of nauplii and copepodites.

### Changes in chlorophyll

Chl *a* at the onset of the experiments was  $0.48 \pm 0.03 \mu g$  Chl *a*  $L^{-1}$  in August 2002 and  $0.98 \pm 0.19 \mu g$  Chl *a*  $L^{-1}$  in March 2003 (Fig. 2). Chl *a* concentrations in the addition treatment of the August experiment were significantly higher after the first 24 h of incubation compared to values at the beginning of the experiment for the same treatment and were significantly higher than the values in the other two treatments at  $T_{24}$  (both  $P < 0.05$ ,  $t$ -test; Fig. 2). However, there were no significant differences in Chl *a* concentrations among the three treatments after 72 h of incubation (Fig. 2). In contrast, Chl *a* during the March experiment did not change significantly in any of the three treatments during the first 24 h of incubation,



**Fig. 2.** Changes in chlorophyll *a* (Chl *a*) during incubations in the absence of metazoa  $> 80 \mu m$  (exclusion treatment), in the presence of natural abundances of metazoa [whole seawater (WSW) treatment] and with elevated abundances of copepods (addition treatment) during August 2002 and March 2003. Means derived from three bottles are shown with their standard deviation. # indicates that the values were significantly different from the values at a previous sampling point for the same treatment ( $P < 0.05$ ,  $t$ -test). § indicates the values were significantly different from the two other treatments at the same time point ( $P < 0.05$ ,  $t$ -test).

but values in the exclusion and WSW treatments were significantly lower after 72 h of incubation relative to concentrations at  $T_0$  and 24 h ( $P < 0.05$ ,  $t$ -test). Chl  $a$  concentration at 72 h in the addition treatment did not differ significantly from values in this treatment at  $T_0$  and 24 h. However, Chl  $a$  concentrations differed significantly among all three treatments after 72 h of incubation ( $P < 0.05$ ,  $t$ -test; Fig. 2).

### Changes in 10–80 $\mu\text{m}$ plankton biomass

Ciliate biomass during the August experiment was significantly lower after 24 h in the addition treatment relative to  $T_0$  and compared to the WSW and exclusion treatment ( $P < 0.05$ ,  $t$ -test; hatched bars in Fig. 3). Ciliate biomasses after 72 h of incubation in both the addition and WSW treatments of that experiment were significantly lower than in the exclusion treatment ( $P < 0.05$ ,  $t$ -test, Fig. 3). In contrast, ciliate biomass increased slightly (but not significantly) throughout the experiment in the bottles from which metazoa were removed (open bars in Fig. 3). Overall, we observed no significant changes in dinoflagellate biomass for either phototrophic or heterotrophic taxa over the course of the 72 h experiment in August (Fig. 3). However, diatom biomass increased significantly over the duration of the experiment in all treatments ( $P < 0.05$ ,  $t$ -test), and the addition and WSW treatments yielded significantly higher diatom abundances than the exclusion treatment ( $P < 0.05$ ,  $t$ -test; Fig. 3). Values for other flagellates  $>10 \mu\text{m}$  in size (non-dinoflagellates) increased in the addition treatment during the first 24 h of the incubation relative to abundances in the other treatments ( $P < 0.05$ ,  $t$ -test; Fig. 3), but no significant differences in biomass were found among the treatments for this category after 72 h. Heterotrophic and phototrophic cells contributed approximately equally to the other flagellate assemblage in August irrespective of treatment or time point (data not shown).

Ciliate biomass during the March experiment did not differ significantly among the treatments during the first 24 h of incubation, but increased significantly after 72 h in the bottles from which metazoan predators were excluded ( $P < 0.05$ ,  $t$ -test, Fig. 3). Ciliate biomass in this exclusion treatment was also significantly greater than in treatments with natural abundances of copepods (WSW) and elevated copepod abundances (addition) after 72 h ( $P < 0.05$ ,  $t$ -test; Fig. 3). Values in these latter treatments after 72 h of incubation were not significantly changed from their initial values. No significant biomass changes were observed for the non-ciliate plankton assemblages in the 10–80  $\mu\text{m}$  size fraction (phototrophic and heterotrophic dinoflagellates, diatoms and other flagellates) over the duration of the experiment or among the different treatments in March (Fig. 3).

### Changes in nano- and picoplankton biomass

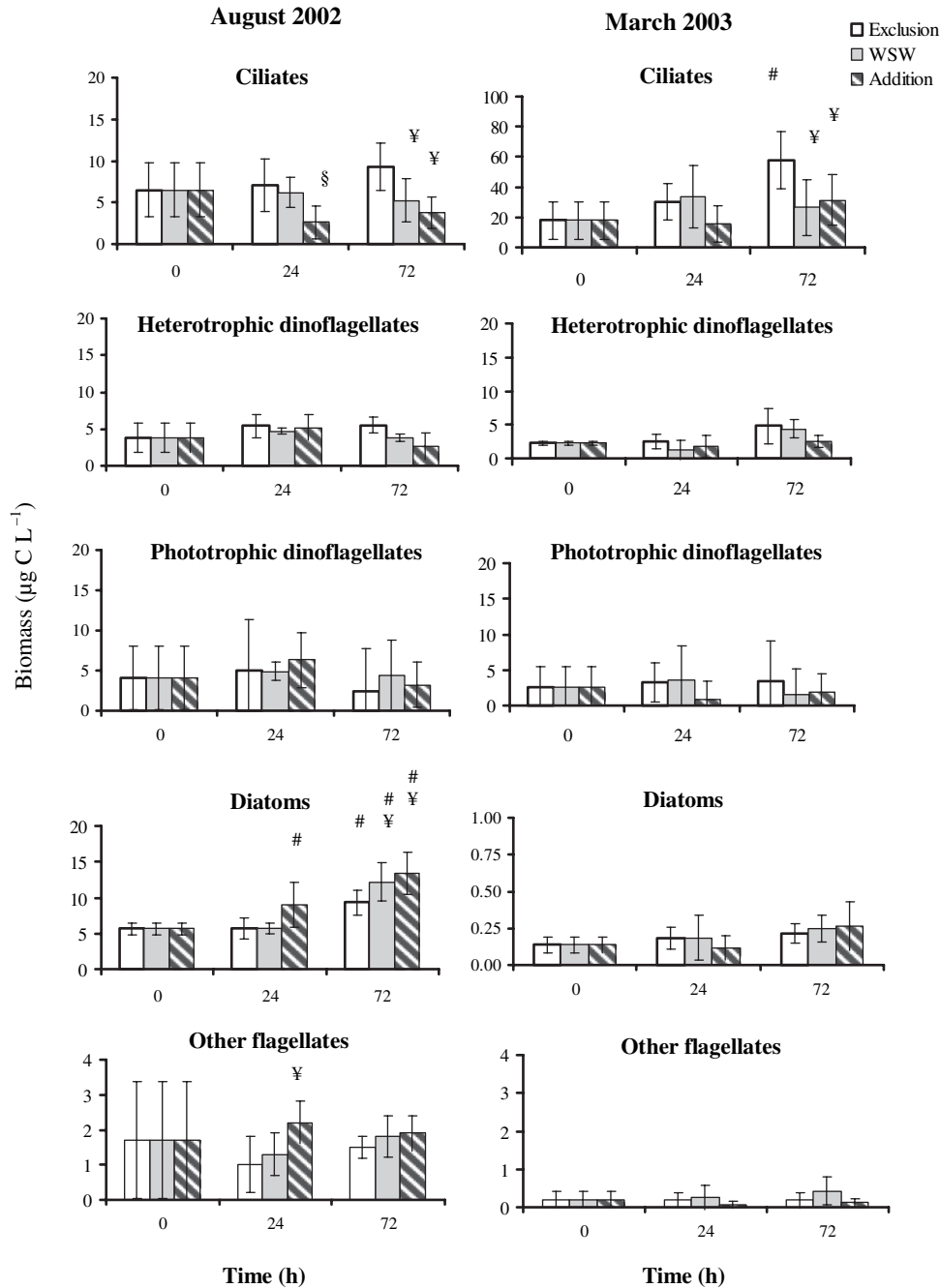
Significant differences in the biomass of eukaryotes  $<10 \mu\text{m}$  were observed among the treatments after 72 h of incubation during the August 2002 experiment, but not after 24 h of incubation (Fig. 4). Values for nanophototrophs (2.5–10  $\mu\text{m}$ ), nanoheterotrophs ( $<10 \mu\text{m}$ ) and phototrophic eukaryotes ( $<2.5 \mu\text{m}$ ) in the addition treatment were significantly greater than values in the exclusion and WSW treatments by the end of the experiment. Changes in the biomass of heterotrophic picoplankton (bacteria + archaea) and phototrophic prokaryotes (*Synechococcus* spp. + *Prochlorococcus* spp.) during the incubations showed no consistent trends among the treatments, although abundances of phototrophic prokaryotes generally decreased throughout the 72-h incubation (Fig. 4).

Only the nanophototroph assemblage (2.5–10  $\mu\text{m}$ ) showed significant differences among treatments for microbial assemblages  $<10 \mu\text{m}$  during the March 2003 experiment (Fig. 4). Nanophototrophic biomass was significantly greater in the WSW and addition treatments relative to the exclusion treatment (both  $P < 0.05$ ,  $t$ -test). The only other significant changes in the  $<10 \mu\text{m}$  assemblages in the March experiment were decreases in all treatments for phototrophic eukaryotes ( $<2.5 \mu\text{m}$ ) and in the exclusion and addition treatment for phototrophic prokaryotes during the 72-h incubations (Fig. 4).

### Copepod ingestion rates

Ingestion rates of copepods for ciliates and heterotrophic dinoflagellates were calculated for both experiments from changes in their abundances. The highest ingestion rates were observed during the initial 24 h in the WSW treatment during the August 2002 experiment (Table II). Copepods ingested  $2.06 \pm 0.60 \mu\text{g C}$  and  $0.78 \pm 0.07 \mu\text{g C copepod}^{-1} \text{ day}^{-1}$  for ciliates and heterotrophic dinoflagellates, respectively. Combined carbon intake for these two assemblages corresponded to a daily ration of 98% body C for that experiment and time frame (Table II). Ingestion rates in the addition treatment of the same experiment were  $0.56 \pm 0.43 \mu\text{g C}$  and  $0.02 \pm 0.01 \mu\text{g C copepod}^{-1} \text{ day}^{-1}$  for ciliates and heterotrophic dinoflagellates during the first 24 h, corresponding to a total daily ration of 20% body C. Ingestion rates in both treatments during August decreased dramatically during the latter part of the experiment ( $T_{24}$ – $T_{72}$ ) to  $0.38 \mu\text{g C}$  (daily ration of 13% body C  $\text{day}^{-1}$ ) and  $0.13 \mu\text{g C copepod}^{-1} \text{ day}^{-1}$  (daily ration of 4% body C  $\text{day}^{-1}$ ). Overall, during August 2002 ciliates constituted 18% (WSW;  $T_0$ – $T_{72}$ ) of the copepod daily ration, while heterotrophic dinoflagellates constituted 7% (Table II).

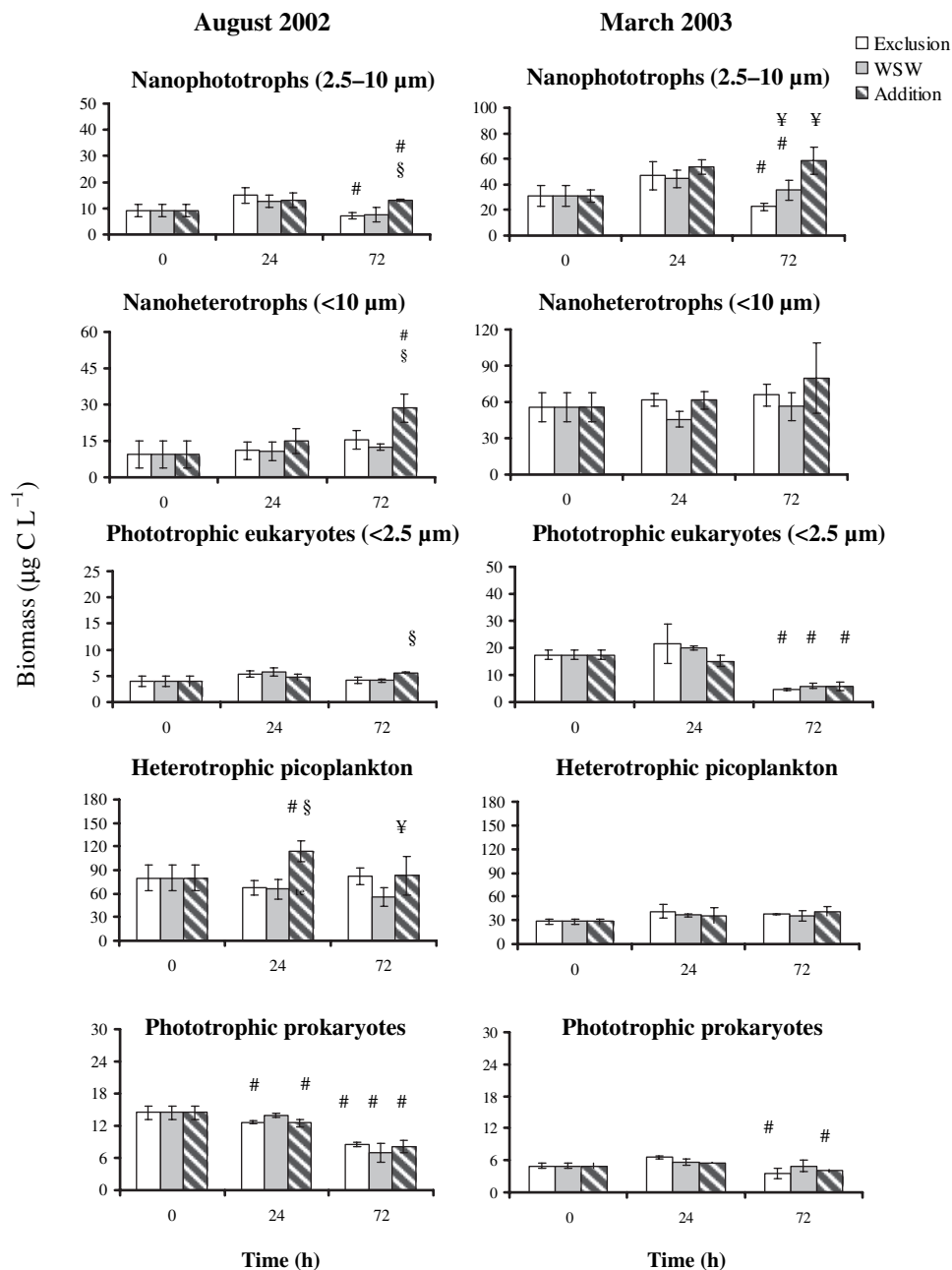
Contributions of ciliate and heterotrophic dinoflagellate biomass to copepod diets during the March



**Fig. 3.** Biomass changes for protistan microplankton (ciliates, heterotrophic and phototrophic dinoflagellates, diatoms and other flagellates) in the 10–80  $\mu\text{m}$  size range during incubations in the absence of metazoa  $> 80 \mu\text{m}$  (exclusion treatment), in the presence of natural abundances of metazoa [whole seawater (WSW) treatment] and with elevated abundances of copepods (addition treatment) during August 2002 and March 2003. Means were derived from three bottles and error bars indicate standard deviation. # indicates that the values were significantly different from the values at a previous sampling point for the same treatment ( $P < 0.05$ , *t*-test). ¥ indicates that the values were significantly different from the values for the exclusion treatment at the same time point ( $P < 0.05$ , *t*-test). § indicates the values were significantly different from the two other treatments at the same time point ( $P < 0.05$ , *t*-test). Note differences in the scale for y-axis.

2003 experiment were less than their contributions during the August experiment, and measurable ingestion rates on these assemblages were not consistently detected during

the 72-h experiment. Copepod ingestion rates for ciliates were undetectable during the first 24 h in the WSW treatment of the March experiment, while ingestion



**Fig. 4.** Biomass changes for phototrophic and heterotrophic nanoplankton (< 10 μm), phototrophic picoeukaryotes, heterotrophic (bacteria + archaea) and phototrophic (*Synechococcus* spp. + *Prochlorococcus* spp.) prokaryotes during incubations in the absence of metazoa > 80 μm (exclusion treatment), in the presence of natural abundances of metazoa [whole seawater (WSW) treatment] and with elevated abundances of copepods (addition treatment) during August 2002 and March 2003. Means were derived from three bottles and error bars indicate standard deviation. # indicates that the values were significantly different from the values at a previous sampling point for the same treatment ( $P < 0.05$ ,  $t$ -test). ¥ indicates values were significantly different from the values for the exclusion treatment at the same time point ( $P < 0.05$ ,  $t$ -test). § indicates the values were significantly different from the two other treatments at the same time point ( $P < 0.05$ ,  $t$ -test). Note differences in the scale for y-axis.

rates during the same period for heterotrophic dinoflagellates were  $0.21 \pm 0.23 \mu\text{g C copepod}^{-1} \text{ day}^{-1}$  (3% body C  $\text{day}^{-1}$ ; Table II). Measurable ingestion rates were observed for ciliates during the latter part of the experiment ( $T_{24}$ - $T_{72}$ ;  $1.43 \pm 1.0 \mu\text{g C copepod}^{-1} \text{ day}^{-1}$

= 20% body C  $\text{day}^{-1}$ ), while ingestion rates for heterotrophic dinoflagellates were undetectable. Ingestion rates for heterotrophic dinoflagellates were measurable but low during the 72-h incubation in the addition treatment ( $0.03$  and  $0.02 \mu\text{g C copepod}^{-1} \text{ day}^{-1}$  = 0.4 and 0.3%



Table II: Carbon ingestion rates ( $\mu\text{g C individual}^{-1} \text{ day}^{-1}$ ) for copepods preying on ciliates and heterotrophic dinoflagellates during feeding experiments in August 2002 and March 2003

	Prey	Ciliates ( $\mu\text{g C individual}^{-1} \text{ day}^{-1}$ )	Ration (% body C)	Heterotrophic dinoflagellates ( $\mu\text{g C individual}^{-1} \text{ day}^{-1}$ )	Ration (% body C)	Total ration (% body C)
WSW treatment (August 2002)	$T_0-T_{24}$	2.06 ( $\pm 0.60$ )	71	0.78 ( $\pm 0.07$ )	27	98
	$T_{24}-T_{72}$	0.25 ( $\pm 0.13$ )	9	0.13 ( $\pm 0.02$ )	4	13
	$T_0-T_{72}$	0.54 ( $\pm 0.27$ )	18	0.21 ( $\pm 0.03$ )	7	25
Addition treatment (August 2002)	$T_0-T_{24}$	0.56 ( $\pm 0.43$ )	19	0.02 ( $\pm 0.01$ )	1	20
	$T_{24}-T_{72}$	–	–	0.13 ( $\pm 0.09$ )	4	4
	$T_0-T_{72}$	0.21 ( $\pm 0.11$ )	7	0.11 ( $\pm 0.07$ )	4	11
WSW treatment (March 2003)	$T_0-T_{24}$	–	–	0.21 ( $\pm 0.23$ )	3	3
	$T_{24}-T_{72}$	1.43 ( $\pm 1.00$ )	20	–	–	20
	$T_0-T_{72}$	1.00 ( $\pm 0.70$ )	14	0.01 ( $\pm 0.22$ )	0.1	14
Addition treatment (March 2003)	$T_0-T_{24}$	0.64 ( $\pm 0.49$ )	9	0.03 ( $\pm 0.03$ )	0.4	9
	$T_{24}-T_{72}$	–	–	0.02 ( $\pm 0.01$ )	0.3	0.3
	$T_0-T_{72}$	0.21 ( $\pm 0.11$ )	3	0.02 ( $\pm 0.01$ )	0.3	3

–, No positive feeding rates were obtained.

Average ingestion rates are listed ( $\pm$ SD) for the initial 24 h ( $T_0-T_{24}$ ), the latter part of the experiment ( $T_{24}-T_{72}$ ) and for the total incubation period of 72 h ( $T_0-T_{72}$ ) for the whole seawater (WSW) treatment (copepods in natural abundance) and the addition treatment (elevated number of copepods). Ingestion rates are also expressed as percent body C ingested  $\text{day}^{-1}$ .

body C  $\text{day}^{-1}$ ), and only detectable during the first 24 h for ciliate ingestion ( $0.67 \mu\text{g C copepod}^{-1} \text{ day}^{-1} = 9\%$  body C  $\text{day}^{-1}$ ). Overall, in the March 2003 experiment ciliates constituted 14% (WSW;  $T_0-T_{72}$ ) of the copepod daily ration, while heterotrophic dinoflagellates constituted 0.1% (Table II).

Estimates of the daily respiratory requirements of copepods were 1.8 and 2.2  $\mu\text{g C copepod}^{-1} \text{ day}^{-1}$  (61 and 32% body C  $\text{day}^{-1}$ ) for August and March, respectively. Based on the ingestion rates of ciliates and heterotrophic dinoflagellates by copepods in the WSW treatments, respiratory requirements of the copepods were fully met via the ingestion of protozooplankton during the first 24 h of the August experiment (total ingestion = 98% body C  $\text{day}^{-1}$ ) but not during the March experiment.

## DISCUSSION

### Copepod predation on microplankton

The contribution of ciliates (dominantly aloricate oligotrichs) to copepod diets in the present study markedly exceeded the contribution of heterotrophic dinoflagellates (combining both experiments  $\sim 81\%$  of the ingested carbon comprised of ciliates; range = 64–99%).

Previous studies have noted that the small copepod genera *Clausocalanus* spp., *Oithona* spp. and *Acartia* spp. that dominated the San Pedro Channel assemblage in August 2002 and March 2003 are known as important consumers of ciliates and dinoflagellates (Stoecker and Egloff, 1987; Mazzocchi and Paffenhöfer, 1999; Turner *et al.*, 2001; Turner, 2004). The raptorial feeding modes of *Oithona* spp. and *Acartia* spp. are suited to sense and capture mobile prey such as ciliates and *Clausocalanus* spp. is well adapted to efficiently capture motile dinoflagellate cells (Gifford and Dagg, 1991; Mazzocchi and Paffenhöfer, 1999; Lonsdale *et al.*, 2000; Granéli and Turner, 2002).

Broglio *et al.* (Broglio *et al.*, 2004) estimated daily carbon intake rates of copepods feeding on ciliates of 24–101% of copepod body C  $\text{day}^{-1}$  over an annual cycle in oligotrophic coastal waters of the Mediterranean Sea. Estimates for the ingestion of protozoa (mainly ciliates) by copepods reported in previous studies fall largely within this wide range, and the higher values were generally reported when phytoplankton assemblages were dominated by cells  $< 5 \mu\text{m}$  (Fessenden and Cowles, 1994; Nakamura *et al.*, 1997; Lonsdale *et al.*, 2000).

In our study the ingestion of ciliates and heterotrophic dinoflagellates in whole unfiltered seawater (WSW)

constituted an average of 25% (maximum of 98%) of copepod body C day<sup>-1</sup> in August 2002 and an average of 14% (maximum of 20%) of copepod body C day<sup>-1</sup> in March 2003. Individual ingestion rates were lower when copepod numbers in the enclosures were increased (addition treatment) compared to natural abundances (WSW treatment), and they generally decreased during the later phase of both feeding experiments ( $T_0$ - $T_{24}$  vs.  $T_{24}$ - $T_{72}$ , Table II).

Mean respiratory requirements of the copepods in the two experiments were estimated with 61% and 32% of the copepod body C day<sup>-1</sup> for August and March, respectively. When compared to the carbon intake calculated from the average ingestion rates for protozooplankton in the August experiment ( $T_0$ - $T_{72}$ ), copepods obtained approximately 41% of their daily respiratory requirements by preying on ciliates and heterotrophic dinoflagellates during the 3-day experiment. The contribution was considerably greater during the period  $T_0$ - $T_{24}$ . Ingestion of protozooplankton during the first 24 h of the August experiment constituted 98% of copepod body C day<sup>-1</sup>, exceeding our estimate for the basic respiratory requirements of the copepods (61% of copepod body C day<sup>-1</sup>). This indicates that the copepods could have sustained themselves entirely by feeding on protozooplankton during that time. Decreases in the abundance of protozooplankton during the latter part of the incubation ( $T_{24}$ - $T_{72}$ ) probably acted to decrease ingestion rates and thus the contribution of ciliates and heterotrophic dinoflagellates to carbon intake by the copepods.

Copepod carbon intake from protozoan prey over the entire length of the March experiment ( $T_0$ - $T_{72}$ ) constituted 44% of their estimated respiratory requirement (intake = 14% copepod body C day<sup>-1</sup>; estimated respiratory requirement = 32% copepod body C day<sup>-1</sup>) and did not exceed respiratory requirements during either phase of the experiment. Thus, the contribution of protozooplankton to overall copepod diets at that time presumably was modest.

Diatom growth in the experimental bottles during August exceeded losses due to grazing pressure, even in the presence of enhanced copepod abundances (Fig. 3). The absence of a significant grazing impact on diatoms may be explained by preferential feeding of copepods on microzooplankton relative to diatoms. This behavior has been noted in previous studies (Stoecker and Egloff, 1987; Stoecker and Capuzzo, 1990; Atkinson, 1996; Verity and Paffenhoefer, 1996; Turner *et al.*, 2001; Broglio *et al.*, 2004). In addition, diatoms may have benefited from a decrease in herbivorous microzooplankton due to copepod predation on the latter species (Levinsen and Nielsen, 2002; Jeong *et al.*, 2004). This

explanation is in agreement with the results of the August experiment in which significant increases in diatom biomass were observed in the treatments with natural abundances and enhanced abundances of copepods (WSW and addition) relative to the treatment with copepods excluded (Fig. 3).

Early studies on development, growth and egg production in copepods promulgated the views that diatoms are a high-quality prey and that they constitute most of the diets of these crustacea. More recently, the benefit of a mixed diet for copepods has been demonstrated (Kleppel, 1993; Bonnet and Carlotti, 2001), and the long-standing belief of the overwhelming importance of diatoms as a food source for copepods has been questioned (Stoecker and Capuzzo, 1990; Gifford and Dagg, 1991; Jónasdóttir *et al.*, 1998; Ianora *et al.*, 2003; Jones and Flynn, 2005). Indeed, field studies indicate that some diatom taxa compromise copepod hatching viability due to the production of reactive aldehydes (Miralto *et al.*, 1999, 2003; Ianora *et al.*, 2004). Miralto *et al.* (Miralto *et al.*, 2003) identified species of the genus *Pseudo-nitzschia* among these aldehyde-producing species. Interestingly, the diatom assemblage from the San Pedro Channel during our experiment in August 2002 was dominated by *Pseudo-nitzschia* spp. when the highest predation rates by copepods on ciliates and heterotrophic dinoflagellates in our experiments were observed.

### Regulation of microzooplankton biomass by copepod grazing

Individual copepod grazing rates for protozooplankton during August 2002 generally exceeded those during March 2003 (Table II). Even so, the overall grazing impact of the copepod community on the protozooplankton assemblage was likely higher in March, since copepod abundances in our samples during March exceeded those in August by 8-fold. Our estimates for *in situ* copepod abundances are approximate because they are based on relatively small sampling volume and do not take into account spatial or temporal patchiness in mesozooplankton distribution. However higher abundances of copepods in spring versus summer have been commonly observed in the San Pedro Channel (San Pedro Ocean Time-series). Applying average copepod ingestion rates from our incubation experiments to the entire copepod community indicated that the proportion of microzooplankton biomass that was removed by the copepod assemblage was modest at 4.6% day<sup>-1</sup> in August 2002, but significant at 36% day<sup>-1</sup> in March 2003 (WSW,  $T_0$ - $T_{72}$ ). The impact of copepod grazing on ciliate standing stocks during August fell within the lower range of previously reported values (<1-25% day<sup>-1</sup>) from a variety of different environments (Fessenden and

Cowles, 1994; Atkinson, 1996; Lonsdale *et al.*, 2000; Zeldis *et al.*, 2002; Broglio *et al.*, 2004), while copepods had a significant impact on resident ciliate populations in March. Despite this high grazing pressure, the ciliates maintained their population size throughout the March incubations even when copepod abundances were increased (addition treatment).

### Trophic cascades

The manipulation of copepod abundances had a direct, measurable effect on ciliate biomass in both experiments conducted in this study. This grazing impact also resulted in an apparent cascade effect on some small (<10  $\mu\text{m}$ ) protistan assemblages which apparently benefited from altered grazing pressure by ciliates and possibly heterotrophic dinoflagellates. A 14-fold enhancement of copepod abundances (addition treatment) during the August 2002 experiment resulted in significant increases in nanoplankton biomass (phototrophs and heterotrophs) and phototrophic eukaryotes <2.5  $\mu\text{m}$  in size relative to the other treatments (Fig. 4). A comparison of the exclusion and WSW treatments during August indicated that natural abundances of copepods in that experiment (<1 individual  $\text{L}^{-1}$ ) were insufficient to result in a detectable trophic cascade on nanoplankton abundances during the 3-day experiment.

In contrast, natural abundances of copepods were 8-fold higher during the March experiment (8 individuals  $\text{L}^{-1}$ ) and resulted in a significant increase in the biomass of phototrophic nanoplankton in WSW enclosures relative to the exclusion treatment. Enhanced abundances of copepods in the addition treatment of this experiment (2.4 $\times$  natural abundance) resulted in an increase in phototrophic nanoflagellate biomass relative to the WSW treatment as well as the exclusion treatment. Changes in Chl *a* concentrations during the March experiment confirmed that phototrophs generally thrived in the presence of copepods (Fig. 2).

We speculated that the prokaryote assemblages in our experiments might display negative relationships to copepod abundances given the generally positive increases of small protistan assemblages observed. This speculation assumes trophic coupling in the order copepods-microzooplankton-nanozooplankton-prokaryotes and an alternating trophic cascade due to the manipulation of copepod abundances. However, responses of phototrophic and heterotrophic prokaryote biomass consistent with our expectations of altered copepod abundances (exclusion, addition) were not observed in either experiments (Fig. 4). Changes in prokaryote biomass were generally similar among the three treatments (with one exception). Trophic coupling between the bacteria and higher trophic levels have been reported

in previous studies (Calbet and Landry, 1999; Sipura *et al.*, 2003), where increases in bacterial abundances were observed in response to increases in copepod abundances. It was speculated in those studies that copepod grazing activity increased the concentration of substrate available for bacterial growth. If copepod grazing lead to elevated levels of remineralized nutrients in WSW and addition bottles it may in part explain diatom growth in August being positively correlated to copepod abundances. Several potentially confounding interactions affect how the manipulation of copepod abundance might translate to microbial assemblages two or three trophic levels removed. Other potentially important aspects that could not be addressed included loss of protozoan biomass due to carnivory within protistan populations or direct trophic interactions that extended over several trophic levels (e.g. bacterivorous ciliates).

The experimental approach taken in this study provides insight into the trophodynamics of the plankton community of the San Pedro Channel. Ciliates constituted an important food source for copepods in experiments conducted during August 2002 and March 2003 and a key trophic link between nanoplankton assemblages and copepod predators. Top-down regulation by copepods appeared to play an important role in maintaining the overall trophic structure of the microbial assemblage in March 2003 where copepod grazing pressure was several-fold higher than in August 2002. Quantitative estimates of abundance and biomass changes of various microbial plankton assemblages and their response to the alteration of copepod abundances improves our overall understanding of plankton trophic dynamics in marine ecosystems. Further (genetic) analyzes of protistan community structure during these experiments (Schnetzer *et al.*, in preparation) will provide detailed information on the role of specific taxa within this coastal ecosystem.

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